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Contribution of Marine Algae to Trihalomethane Production in Chlorinated Estuarine Water

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16. ABSTRACT

Three species of marine algae representing major taxonomic groups of phytoplankton, Isochrysis galbana (Chrysophyceae), Carteria sp. (Chlorophyceae), and Thalassiosira pseudonana (Bacillariphyceae), were utilized to investigate the potential of natural occuring chlorophyll a of living algae to produce trihalomethanes during the chlorination of saline waters. Chlorination of filtered natural estuarine water (salinity-23 p.p.t.) from the North Edisto River, South Carolina, results in rapid formation of 201-221 ug l^{-1} trihalomethanes comprised mainly of bromoform ($CHBr_3$) and chlorodibromomethane ($CHBr_2Cl$). In the presence of 10^6 cells ml^{-1} Isochrysis galbana, chlorination of filtered estuarine water with sodium hypochlorite (NaOCl) to a nominal 10 mg l^{-1} chlorine increased the total trihalomethane concentration by an average of 41 % (N=6). The presence of Thalassiosira pseudonana resulted in an average 24 % decrease (N=6) while Carteria sp. did not produce a statistically significant effect upon the total trihalomethane concentration formed.

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Contribution of Marine Algae to Trihalomethane Production in Chlorinated Estuarine Water

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Three species of marine algae representing major taxonomic groups of phytoplankton, *Isochrysis galbana* (Chrysophyceae), *Carteria* sp. (Chlorophyceae), and *Thalassiosira pseudonana* (Bacillariophyceae), were utilized to investigate the potential of naturally occurring chlorophyll *a* of living algae to produce trihalomethanes during the chlorination of saline waters. Chlorination of filtered natural estuarine water (salinity = 23 p.p.t.) from the North Edisto River, South Carolina, results in rapid formation of 201–221 μg l^{-1} trihalomethanes comprised mainly of bromoform ($CHBr_3$) and chlorodibromomethane ($CHBr_2Cl$). In the presence of 10^8 cells ml^{-1} *Isochrysis galbana*, chlorination of filtered estuarine water with sodium hypochlorite (NaOCl) to a nominal 10 mg l^{-1} chlorine increased the total trihalomethane concentration by an average of 41% ($N=6$). The presence of *Thalassiosira pseudonana* resulted in an average 24% decrease ($N=6$) while *Carteria* sp. did not produce a statistically significant effect upon the total trihalomethane concentration formed.

The absence of any significant statistical correlations between the chlorophyll *a* content of algal cultures and trihalomethane concentrations causes us to discount chlorine–chlorophyll *a* interactions as a source of these compounds. However, trihalomethane concentrations produced from the chlorination of algal culture media, after removal of algal populations, suggests instead that the observed trends in trihalomethane production are mainly due to chlorines reaction with the by-products of algal metabolism.

Introduction

The production of trihalomethanes from the chlorination of both fresh waters (Jolley *et al.*, 1975; Keith *et al.*, 1976; Kleopfer, 1976) and marine waters (Bean *et al.*, 1978; Helz & Hsu, 1978) has lead to continuing investigations into the identity of the natural precursors of these compounds (Rook, 1974, 1977; Stevens *et al.*, 1975). Baum & Morris's (1978) study of the molecular structures of potential haloform precursors indicated that the pyrrole ring, a basic component of the porphyrin skeleton of chlorophyll, may undergo chlorination resulting in formation of chloroform ($CHCl_3$). In a preliminary test of this hypothesis,

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Baum & Morris (1978) dosed a chlorophyllin preparation with 40 mg l^{-1} of chlorine as sodium hypochlorite (NaOCl). After 100 h contact, substantial amounts of $CHCl_3$ were recovered, particularly if the reaction medium was basic: at pH of 7·0, 46 µg l^{-1} of $CHCl_3$ was produced, whereas at pH 9·2, 260 µg l^{-1} was detected.

The apparent ability of the pyrrole rings of chlorophyllins to generate $CHCl_3$ when dosed with aqueous chlorine implies that the pyrrole rings of chlorophyll in marine algae might also act as natural precursors for trihalomethanes. Fundamental differences, however, between the chemical environment of the pyrrole rings of water-soluble chlorophyllins and insoluble chlorophyll, the latter being contained in the chloroplast of living algal cells, requires that analogies between the chemical reactivity of the two systems be confirmed experimentally. We therefore initiated the following investigation to determine the extent chlorophyll *a* of living marine algae contributes to the production of trihalomethanes during the chlorination of estuarine water.

TABLE 1. Composition of enrichment added to algal culture medium

$NaNO_3$	0·1 g l^{-1}
K_2HPO_4	0·01 g l^{-1}
$NaHCO_3$	0·2 g l^{-1}
Metals mixture[a]	1·0 ml
Vitamin mixture[b]	1·0 ml

[a]One millilitre contained: Fe (as $FeCl_3.6H_2O$), 0·25 mg; Mn (as $MnCl.4H_2O$), 0·1 mg; Zn (as $ZnCl_2$) 0·01 mg; and Co (as $CoCl_2.6H_2O$), 0·001 mg.
[b]One millilitre contains: thiamine hydrochloride, 5 mg; vitamin B_{12}, 0·5 µg; and biotin, 0·5 µg.

Materials and methods

Algal sample preparation

Isochrysis galbana, *Thalassiosira pseudonana* (obtained from culture collection, Dr R. Steele, USEPA, Environmental Research Laboratory, Narragansett, R.I.) and *Carteria* sp. cultures (obtained from W. B. Wilson, Moody College of Marine Sciences and Maritime Resources, Texas A & M University, Galvaston, Texas) were grown in estuarine water collected from the North Edisto River (T=10 °C, S=23 p.p.t., pH=7·9, turbidity=16 formazin turbidity units, ammonium N<0·01 p.p.m.) at the USEPA Bears Bluff Field Station, Wadmalaw Island, South Carolina. Sample water was immediately filtered through a prewashed (Parker, 1967) 0·22 µm Millipore filter to remove natural plankton populations, and then supplemented with growth nutrients (Erickson *et al.*, 1970) (Table 1).

Cultures were incubated in 18 l glass carboys in an illuminated constant temperature chamber (Precision Scientific Model 818) maintained at 20 °C±1 °C. Incident illumination of 2690 lx cool-white fluorescent light was supplied continuously.

To ensure a maximum concentration of algal cells, all experimental cultures were allowed to attain their stationary growth phase prior to being used. Stationary growth, as defined by an absence of logarithmic growth for 12 h, was determined by algal cell counts with a Model Z_B Coulter particle counter, while the cell volume of cultures was determined by an RBC/MCV/Hct. computer interfaced with the particle counter (Coulter Electronics Inc.).

Nutrient-enriched estuarine water was used as uninoculated control blanks and as dilution medium. Blanks were maintained under the same light and temperature conditions as algal cultures. Cultures and blanks were adjusted to pH 8·0±0·05 (Corning Model 7 pH meter) with 0·1 N NaOH and 0·1 N HCl before exposure to NaOCl solutions.

Estuarine water and trihalomethane production

Filtered estuarine water blanks were first examined for a capacity to produce trihalomethanes when chlorinated in the absence of algae. After equilibration to room temperature (20 °C), sample water was adjusted to pH 8 and subdivided into eighteen 100 ml glass volumetric flasks. Triplicate flasks were treated with aqueous NaOCl (Fisher Scientific Co.) to a nominal 10, 20, 30, 40, 50, and 100 mg l^{-1} chlorine. Each flask was stoppered with a Teflon-lined screw cap and left undisturbed for 24 h under overhead fluorescent lighting (2500 lx) at 20 °C, after which residual oxidants were quenched by adding 1 ml of 5% solution of sodium thiosulphate ($Na_2S_2O_3 . 5H_2O$) (Baker Analysed Reagent). Contents of the flasks were immediately extracted using the method reported by Henderson *et al.* (1976). After 2 ml aliquots of distilled-in-glass pentane (Burdick & Jackson Laboratories, Inc.) were pipetted into each flask, the water/pentane mixture was vigorously shaken for approximately 5 min and the two immiscible liquids then allowed to separate completely. A 1·0 µl aliquot of the organic layer was then removed with a microliter syringe for gas chromatographic analysis.

The recovery rate from six fortified samples prepared from the trihalomethanes in ethanol averaged 83% for bromoform ($CHBr_3$) and 85% for chlorodibromomethane ($CHBr_2Cl$), with an average coefficient of variation of 7·4%.

Gas chromatograms were obtained with a Hewlett Packard Model 5710A equipped with a ^{63}Ni electron capture detector. A 1·8 m × 2 mm i.d. glass column, packed with 12% OV-101 on Anakrom Q 100/120 mesh (Analabs, Inc.), was utilized. Injection port and detector temperatures were 150 °C. The column was maintained at 70 °C with 95:5 argon/methane mixture as the carrier gas (40 ml min^{-1}).

$CHBr_3$ and $CHBr_2Cl$ assignments for the two major chromatographic peaks observed were obtained by employing the admixture technique to an extracted sample of chlorinated estuarine water. The resulting chromatograms indicated no additional peaks or shoulders or irregularities on the $CHBr_3$ and $CHBr_2Cl$ peaks. An increase in peak height without corresponding increase in peak width indicated excellent retention coincidence between $CHBr_3$ and $CHBr_2Cl$ standards and the unknowns.

Chlorophyll a and trihalomethane production

To evaluate the potential influence of algal chlorophyll *a* on concentration of trihalomethanes produced during the chlorination of estuarine water three species of marine algae were selected: *I. galbana*, *Carteria* sp., and *T. pseudonana*. Three individually grown cultures of each species were subdivided into four 100 ml volumetric flasks, cells from two of each set of flasks were filtered out and the chlorophyll *a* content of the cells determined according to the method of Strickland & Parsons (1968). The extinctions of chlorophyll *a* at 6650, 6450, and 6300 Å were measured on a Model 2400 Beckman DU spectrophotometer. The filtrate was then returned to clean flasks for chlorination with NaOCl concurrently with flasks containing algal cells. The 12 flasks of each species were exposed to the nominal chlorine concentration of 10 mg l^{-1}. After a contact time of 1 h at 20 °C, the residual oxidants were quenched with 1 ml of a 5% solution of $Na_2S_2O_3 . 5H_2O$. Trihalomethanes produced in each flask were extracted in pentane and quantified according to the method described above.

Trihalomethane production and chlorine demand

The determination of chlorine residuals, when estuarine water with a known algal cell concentration and volume is subjected to various chlorine concentrations and contact times,

in conjunction with determinations in estuarine water devoid of any algal cell populations, allowed an estimation of the correlation between trihalomethane production and the amount of chlorine consumed (chlorine demand) by the algal culture itself. The procedure was as follows. For each of three chlorine dose concentrations, portions of stock culture were decanted into twelve 100 ml glass volumetric flasks and six 1 l glass volumetric flasks after reaching a stationary growth phase in approximately 6 days. These subsamples, along with matching subsamples of uninoculated culture media, were exposed to aqueous NaOCl at nominal concentrations of 5, 10 or 20 mg l^{-1} chlorine. After 10 min, the contents of one 1 l flask of each chlorine dose were treated with a solution of 1 ml acetate buffer (pH 4) and 5 ml of 20% potassium iodide (KI), followed by amperometric determination (in triplicate), of total residual oxidant as given in *Standard Methods* (American Public Health Association, 1971). Total residual oxidant determinations were performed using a Wallace and Tiernan amperometric titrator. Phenylarsine oxide titrant (0·0056 N) and acetate buffer solution (pH 4) were obtained from Wallace and Tiernan Company. The 20% KI solution was prepared in our laboratory with 'chlorine demand free' water and reagent grade KI (Fisher Scientific Co.). Rapid titration of each sample (less than 2 min) with minimum agitation helped minimize errors due to volatilization losses of iodine (Manabe, 1974). Two 100 ml volumetric flasks containing algal cells and two containing the blank of uninoculated culture media were extracted with pentane at the end of the 10 min exposure and the extract immediately analysed for trihalomethanes according to the method previously described.

This sequence of events was repeated at 30, 60, 120, and 240 min, and finally on the four remaining 100 ml and the one 1 l flask after 24 h. Constant temperature (20 °C) and lighting (2690 lx) were maintained throughout the experiment as previously outlined.

Results

Estuarine water and trihalomethane production

After 24 h, the total $CHBr_3$ and $CHBr_2Cl$ concentration yield of natural estuarine water chlorinated between a nominal 10 and 100 mg l^{-1} chlorine remained essentially constant,

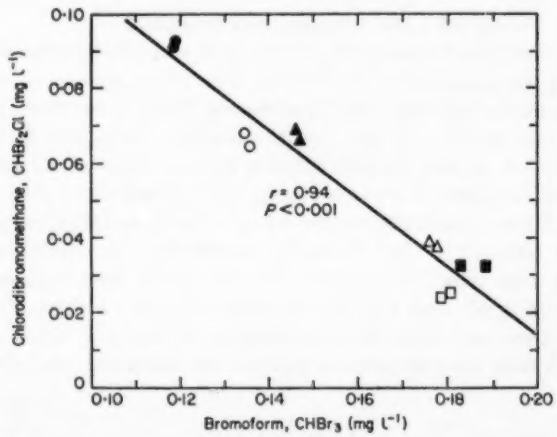

Figure 1. Linear regression for $CHBr_2Cl$ concentration as a function of $CHBr_3$ concentration ($N=18$) in estuarine water dosed with NaOCl to 10 mg l^{-1} (□), 20 mg l^{-1} (■), 30 mg l^{-1} (△), 40 mg l^{-1} (▲), 50 mg l^{-1} (○), and 100 ml l^{-1} (●) nominal chlorine. r is the correlation coefficient. P equals the probability.

averaging 211±8 μg l^{-1} (N=18). Considered individually, however, the concentrations of these two components, comprising total trihalomethane, exhibited opposite relationships to nominal chlorine concentrations: while the concentration of $CHBr_2Cl$ increased with increasing chlorine concentration, $CHBr_3$ was inversely related, decreasing from a high of 188 μg l^{-1} at a nominal 20 mg l^{-1} chlorine to 119 μg l^{-1} at 100 mg l^{-1} chlorine. The increasing concentration of $CHBr_2Cl$ and decreasing $CHBr_3$ concentration exhibited a significant (r=0·94, P<0·001) statistical linear correlation at the 95% confidence level (Figure 1).

Chlorophyll a and trihalomethane production

After exposure to 10 mg l^{-1} chlorine, only one of the three algal species tested, *I. galbana*, significantly increased total trihalomethane concentrations over that produced by the culture medium (Tables 2 and 3). An average 53 μg l^{-1} (N=6) or 41% increase in total trihalomethanes was observed when estuarine water containing approximately 10^6 cells ml^{-1} of this species was chlorinated. *Carteria* sp. had no statistically significant (t test, N=6, α=0·05) effect upon the trihalomethane concentration. Chlorination of approximately 10^5 cells ml^{-1} of *T. pseudonana* resulted in a 50 μg l^{-1} (N=6) or 24% decrease in total trihalomethane production.

Regression analysis of the six replicates of each algal species revealed no significant correlations between the total trihalomethane concentration generated during chlorination and chlorophyll *a* concentrations. Correlation coefficients were less than 0·1 for chlorophyll *a* and total trihalomethane concentrations for all three species of algae.

As is evident in Table 3, chlorination of filtered estuarine water which had previously contained *I. galbana* produced an average 7% increase (N=6) in trihalomethane concentration over that of culture water blanks, but produced significantly less (t test, N=6, α=0·05) than concentrations observed when algal cells were present. In contrast, estuarine

TABLE 2. Characterization of algal populations used in chlorination experiments

Algal species	N	Cell concentration (cells ml^{-1})	Cell volume (μm^3)	Chlorophyll *a* concentration (μg l^{-1})
Isochrysis galbana	6	$1{\cdot}16\times10^6$	$7{\cdot}48\times10^6$	192±10
Carteria sp.	6	$7{\cdot}22\times10^4$	$1{\cdot}22\times10^6$	146±27
Thalassiosira pseudonana	6	$9{\cdot}21\times10^5$	$4{\cdot}77\times10^4$	148±9

N = number of replicates.

TABLE 3. Comparison of average total trihalomethane (THM) concentrations resulting from chlorination of culture medium with and without algal cells. Nominal concentration of chlorine was 10 mg l^{-1}; exposure for 1 h

Algal species	N	THM in culture medium with cells present (μg l^{-1})	% Control	THM in culture medium with cells removed (μg l^{-1})	% Control
Isochrysis galbana	6	181±19	141	125±25	107
Carteria sp.	6	225±13	104	215±25	104
Thalassiosira pseudonana	6	160±16	76	165±35	81

N = number of replicates.

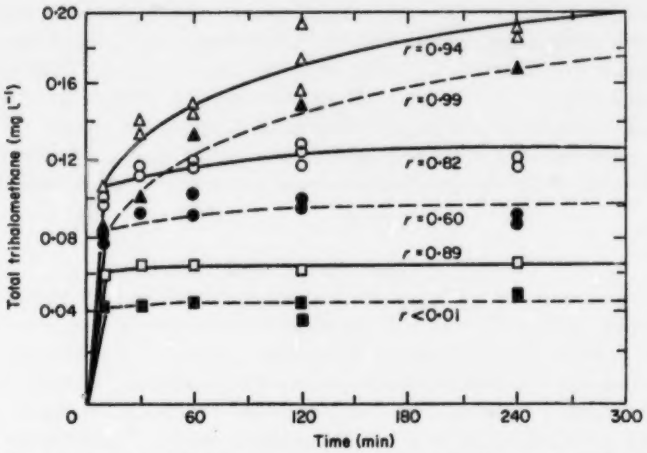

Figure 2. Total trihalomethane production as a function of time for estuarine water without algal cells (– – –) and estuarine water containing 10^6 cells/ml of *Isochrysis galbana* (——), at concentrations of 5 mg l^{-1} (■, □), 10 mg l^{-1} (●, ○), and 20 mg l^{-1} (△, ▲) nominal chlorine. Regression lines for $y=ax^b$ power functions. r is the correlation coefficient.

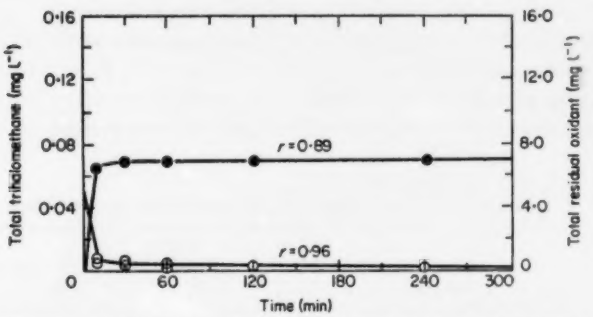

Figure 3. Total trihalomethane (●) and total residual oxidant (○) as a function of time for estuarine water containing 10^6 cells ml^{-1} of *Isochrysis galbana* dosed with aqueous NaOCl to 5 mg l^{-1} chlorine at time zero. r is the correlation coefficient.

water inoculated with *T. pseudonana*, then filtered free of cells, demonstrated a 19% decrease in trihalomethane concentration. This was not statistically different (t test, $N=6$, $\alpha=0{\cdot}05$) from the decreased trihalomethane production of water in which *T. pseudonana* cells were present during chlorination.

Trihalomethane production and chlorine demand

Because only *I. galbana* experimentally demonstrated an apparent capacity to enhance trihalomethane concentrations of chlorinated estuarine water (Table 3), the analysis of trihalomethane production and chlorine demand was restricted to this species. Regression analysis indicated that total trihalomethane production as a function of time is best described by the equation $y=ax^b$, where a equals the y intercept, b is the slope, y equals the concentration of total trihalomethanes (μg l^{-1}), and x is time in minutes. Regression lines

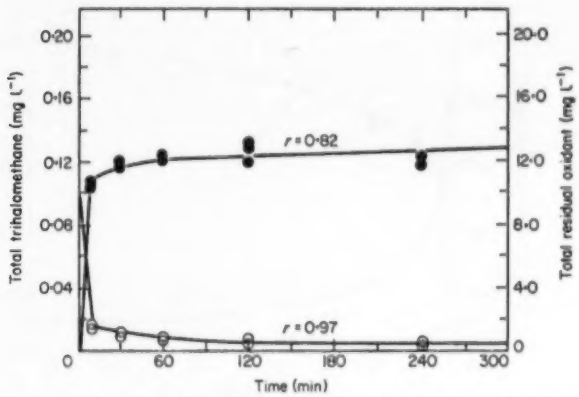

Figure 4. Total trihalomethane (●) and total residual oxidant (○) as a function of time for estuarine water containing 10^6 cells ml^{-1} of *Isochrysis galbana* dosed with aqueous NaOCl to 10 mg l^{-1} chlorine at time zero. r is the correlation coefficient.

TABLE 4. Power function, $y=ax^b$, relationship of total trihalomethane production with time for estuarine water containing 10^6 cells ml^{-1} of *Isochrysis galbana* and cell-free estuarine water dosed with aqueous NaOCl

Sample		Nominal chlorine concentration 5 mg l^{-1}	10 mg l^{-1}	20 mg l^{-1}
Estuarine water with cells	*a*	6·539	7·922	7·496
	b	0·013	0·045	0·171
	r	0·89	0·82	0·94
Estuarine water without cells	*a*	4·466	9·749	5·285
	b	0·027	0·049	0·211
	r	0·10	0·60	0·99

a = y intercept; b = slope; r = correlation coefficient.

for 5, 10, and 20 mg l^{-1} of chlorine are shown in Figure 2 for both filtered estuarine water and estuarine water containing 10^6 cells ml^{-1} of *I. galbana*. Chlorination of filtered water containing cultered *I. galbana* resulted in an increase in total trihalomethane concentration over cell-free estuarine water of 25 ± 3 µg l^{-1} at all three chlorine concentrations. Statistical comparison (F test, $\alpha=0{\cdot}05$) of the differences between regression lines at each concentration confirmed that the observed increases in trihalomethane concentration in estuarine water containing algal populations were significantly greater than blank estuarine water.

In the first 10 min of chlorination the rapid production of trihalomethanes is mirrored by an equally rapid reduction of residual oxidant. As the available oxidant is consumed and its concentration approaches the lower limit of detectability (0·01 mg l^{-1}), no further increases in trihalomethane concentration were observed. At 5 mg l^{-1} and 10 mg l^{-1} chlorine, trihalomethane production rates were not statistically different (Figures 3 and 4) as defined by the slope b of the regression line (Table 4). A similar pattern of rapid initial chlorine reduction accompanied by the production of trihalomethanes is evident at the nominal chlorine dose of 20 mg l^{-1} (Figure 5). However, at this chlorine concentration a persistent residual oxidant continued to produce trihalomethanes for up to 24 h after the initial decline in residual chlorine. Possible influences of algal populations on the chlorine demand of estuarine water were not discernible at nominal 5 mg l^{-1} and 10 mg l^{-1} of chlorine due

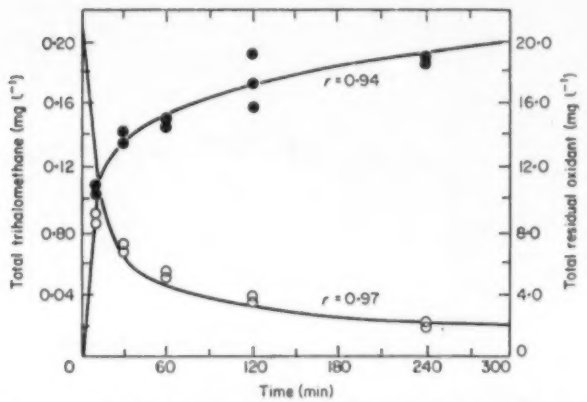

Figure 5. Total trihalomethane (●) and total residual oxidant (○) as a function of time for estuarine water containing 10^6 cells ml^{-1} of *Isochrysis galbana* dosed with aqueous NaOCl to 20 mg l^{-1} chlorine at time zero. r is the correlation coefficient.

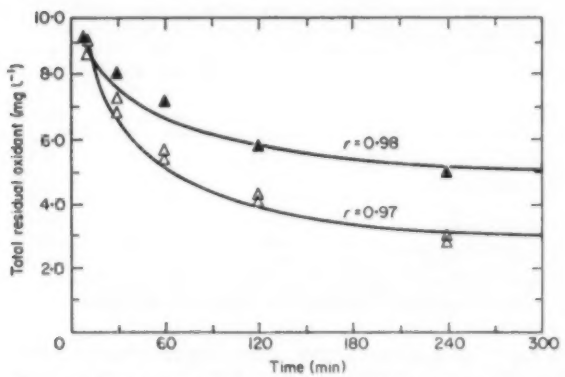

Figure 6. Total residual oxidant as a function of time for estuarine water without algal populations (▲) and with 10^6 cells ml^{-1} of *Isochrysis galbana* (△) at a nominal concentration of 20 mg l^{-1} chlorine. r is the correlation coefficient.

to the rapid reduction of chlorine to nondetectable levels. Differences in chlorine demand between estuarine water containing algal cultures and cell-free water became apparent only at the nominal 20 mg l^{-1} chlorine. At this concentration of chlorine the presence of 10^6 cells ml^{-1} of *I. galbana* accounted for approximately 12% of the residual oxidant consumed after 240 min (Figure 6).

Discussion

In marine waters, naturally occurring bromide is rapidly oxidized by chlorine to hypobromous acid (Johannesson, 1955; Dove, 1970), thus the major halogen constituent of the reaction products resulting from chlorination is no longer the chlorine atom, but bromine. As with $CHCl_3$ in freshwater systems, the bromine analog ($CHBr_3$) is the dominant trihalomethane formed in saline water.

Helz & Hsu (1978) found $CHBr_3$ to be virtually the only trihalomethane produced when chlorine doses of 1–10 mg l^{-1} were applied to filtered estuarine and coastal water samples above 5 p.p.t. salinity from the Patuxent estuary in Maryland. Bean *et al.* (1978) reported that addition of 2 mg l^{-1} NaOCl to water from Sequim Bay, Washington, produced approximately 30 μg l^{-1} $CHBr_3$, smaller quantities of $CHBr_2Cl$, and traces of $CHBrCl_2$. Chlorination up to a nominal 10 mg l^{-1} chlorine of water from the North Edisto River, South Carolina resulted in similar relative concentrations of $CHBr_3$ and $CHBr_2Cl$ being generated (Figure 1). Of the 206 μg l^{-1} total trihalomethanes detected, 180 μg l^{-1} was $CHBr_3$. Noteworthy, however, was our observation of the decreasing $CHBr_3$ and increasing $CHBr_2Cl$ concentrations which accompanied increasing nominal chlorine concentrations beyond 10 mg l^{-1}.

These data suggest that once the chlorine demand of the water is satisfied, residual concentrations of unreacted chlorine may influence the reaction products in one of two possible ways: (1) high chlorine residuals increase the chlorine-to-bromine ratio to a point at which the increased availability of chlorine shifts the competition between chlorine and bromine oxidants in a direction that favors the formation of $CHBr_2Cl$ over $CHBr_3$; (2) the high residual concentration satisfies reactions which normally remove oxidative chlorine from the system (Helz & Hsu, 1978). The available chlorine is then free to attack the previously produced $CHBr_3$ to yield additional $CHBr_2Cl$ while reducing the $CHBr_3$ concentration. However, in our effort to verify this latter possibility, chlorination of $CHBr_3$ standards in distilled water failed to produce any $CHBr_2Cl$ over 24 h.

Production of $CHBr_3$ and $CHBr_2Cl$ by the chemical interaction of bromine with natural organics in water is well documented. Rook (1974) proposed that natural humic substances act as precursors for haloforms. More recent work by Stevens *et al.* (1975) and Rook (1977) indicates that the dihydroxybenzene backbone of humic and fulvic acids may have a substantial influence on haloform production.

In addition to the humates, fulvates, and their degradation products, a large number of other organic compounds present in natural waters have been shown to possess the ability to react in a variety of ways with strong chlorine oxidants. By way of fast ionization to give carbanions, the structures of many of these compounds, including the pyrrole ring, provide sites for chlorination, followed by the formation of trihalomethanes (Baum & Morris, 1978).

However, we did not detect a statistically significant correlation ($r<0{\cdot}1$, $N=18$) between the chlorophyll *a* concentration of algal populations and the production of trihalomethanes from chlorination. This indicates that in these living systems the pyrrole ring does not appear to be a major contributor to the observed production of trihalomethanes. Further, the capacity of culture media, after removal of algal populations, to generate trihalomethane concentrations similar to when algal cells were present strongly suggests that metabolic by-products may have a major influence on the production of trihalomethanes, which explains the variations in trihalomethane concentrations between algal species.

The metabolic material of algae has been shown to cover a broad spectrum of compounds. Guillard & Wangersky (1958) identified a number of carbohydrates as by-products of several marine flagellates. Wangersky & Guillard (1960) reported an organic base as a by-product of a marine dinoflagellate, while Stewart (1963) found amino acid and peptide by-products from marine blue-green algae. Considerable quantities of amino acids and peptides were described as excretory products of many of the algae studied by Hellebust (1965), who also reported that some species excrete a single substance almost exclusively. Many of the major functional groups of these extracellular materials are capable of interacting chemically with chlorine. Chlorination of the carboxylic and hydroxyl functions of

glycolic acid, the nitrogen-containing five-membered ring of proline together with its carboxyl group, and the —OH functions of the common algal by-products glycerol and mannitol should theoretically produce a variety of possible products including the trihalomethanes.

In a preliminary experiment (A. M. Crane, P. K. Kovacic & E. D. Kovacic, unpublished data) chlorination of a number of the algal by-products identified by Hellebust (1965) resulted in variations of trihalomethane production similar to those we observed with living algal cultures. Chlorination of L-proline, for example, resulted in a rapid reduction of residual oxidant without a concurrent production of trihalomethane. Addition of L-proline to a system which generated trihalomethanes upon chlorination caused significant reduction in the trihalomethane concentration normally produced. Conversely, glycolic acid, which is reported to comprise 20% of the total low molecular weight excretion of most algal species (Jüttner & Matuschek, 1978), showed an ability to produce $CHCl_3$ when chlorinated in deionized water, while chlorination of glycerol had no effect on chlorine demand or the production of trihalomethanes.

Summary and conclusions

The important features shown by the data presented in this study are summarized below:

(1) The major trihalomethanes produced by chlorination of estuarine water are $CHBr_3$ and $CHBr_2Cl$. The ratio of $CHBr_3$ to $CHBr_2Cl$ decreases with increasing nominal chlorine concentrations, however, above 10 mg l^{-1} chlorine the total concentration of these two compounds remains constant.

(2) The rate of total trihalomethane production is inversely related in two distinct phases to the reduction rate of chlorination oxidants: an initial rapid production, which goes to completion if the nominal chlorine concentration is below the chlorine demand of the system, followed by a reduced but prolonged production rate if sufficient chlorine is present to maintain a residual oxidant.

(3) The total concentration of trihalomethanes produced by chlorination of estuarine water may, depending upon species, be enhanced or reduced by marine algae, Lack of a statistically significant correlation between chlorophyll *a* concentrations of the phytoplankton populations of this study and trihalomethane concentrations causes us to discount chlorine–chlorophyll *a* interaction as a source of these compounds.

(4) Chlorination of algal culture media, after removal of the cells, generates trihalomethane concentrations similar to when these cells are present. This, together with our results from chlorination of known algal exudates, indicates the influence of algae on trihalomethane concentrations from chlorinated estuarine water is from chlorines reaction with algal by-products.

(5) More detailed experiments addressing the influences of extracellular products of algal metabolism on the chemistry of chlorinated aquatic systems may contribute to a better understanding of the correlation between chlorination and occurrance of volatile halocarbons in both fresh and marine waters.

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